

BIOLOGICAL BULLETIN

ON THE REVERSIBILITY OF THE HELIOTROPISM OF ARENICOLA LARVÆ BY CHEMICALS.¹

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CONTENTS.

I. Introductory.....	149
II. Material and Methods.....	150
III. Experimental.....	151
1. Effects of Temperature Changes.....	151
2. Effects of Hypertonic and Hypotonic Sea-water.....	152
3. Effects of Isotonic Salt Solutions.....	153
4. Effects of Artificial Sea-water.....	155
5. Effects of Hypertonic Sodium and Potassium Chloride Solutions added to the Artificial Sea-water.....	156
6. Effects of Elimination of Salts in the Artificial Sea-water.....	156
7. Effects of Alcohols.....	158
8. Effect of Saponin.....	160
9. Effects of Fatty Acids.....	160
10. "Antagonism" between Acid and Narcotics.....	162
11. Effect of Butyric Acid in Solution with Artificial Sea-water.....	162
12. Effects of Inorganic Acids.....	162
13. Effects of Strong and Weak Bases.....	163
14. Effects of Chemicals after Returning into Normal Sea-water.....	164
IV. Summary.....	165

I. INTRODUCTORY.

Loeb discovered in 1893 that the normal heliotropism of *Polygordius* larvæ and marine copepods could be reversed by changes of the temperature and of the concentration of sea-water.² He also found in 1904 that fresh-water crustaceans which were naturally negative or indifferent to light could be made

¹ From the Marine Biological Laboratory, Woods Hole, Mass., and the Physiological Laboratory, University of Minnesota, Minneapolis.

² Loeb, Jacques, *Pflüger's Arch.*, Bd. 53, p. 81, 1893.

positively heliotropic by chemicals.¹ Since then, Holmes,² Minkiewicz,³ Drzewina,⁴ Jackson,⁵ Moore,⁶ Ewald,⁷ and others have shown many examples of the reversibility of heliotropism in animals by chemicals, temperature, etc. R. S. Lillie,⁸ Mast,⁹ and the writer¹⁰ have also observed the reversing effect of chemicals on the positive heliotropism of *Arenicola* larvæ. These observations, however, were rather incidental and not extensive,

In this paper, the writer shows quantitatively that the normal heliotropism of *Arenicola* larvæ can be reversed by various chemicals and by higher or lower temperatures than normal. And it will be seen that those chemicals which produce artificial parthenogenesis in sea-urchin and other eggs have also a reversing effect on the heliotropism of *Arenicola* larvæ. This parallelism between the reversal of the normal heliotropism of the larvæ and artificial parthenogenesis is striking as regards all chemicals used by the writer except inorganic acids.

The experimental work was done partly under the direction of Professor R. S. Lillie in the physiological department of the Marine Biological Laboratory at Woods Hole, Mass., U. S. A., during the summer of 1914. Publication has been delayed chiefly on account of the writer's return to Japan. The writer wishes here to acknowledge his indebtedness to Prof. Ralph S. Lillie and Prof. Elias P. Lyon for their valuable suggestions and criticism of the work and manuscript. His thanks are due also to Director Frank R. Lillie, who gave him the use of a research room in the laboratory.

II. MATERIAL AND METHODS.

The larvæ of the marine annelid, *Arenicola cristata*, were used for this work. The procedure of obtaining the larvæ was exactly

¹ Loeb, Jacques, Univ. of Calif. Pub., Physiol., Vol. 2, p. 1, 1904.

² Holmes, Samuel J., *Jour. Comp. Neur. and Psychol.*, Vol. 15, 305, 1905.

³ Minkiewicz, R., *Arch. d. Zoöl. Exp. et Gén.*, t. 7, p. 37, 1907.

⁴ Drzewina, Anna, *C. R. Soc. Biol.*, Vol. 71, 555.

⁵ Jackson, H. H. T., *Jour. Comp. Neur. and Psychol.*, Vol. 20, p. 259, 1910.

⁶ Moore, A. R., *Science*, N. S., Vol. 38, p. 131.

⁷ Ewald, Wolfgang F., *Jour. Exp. Zoöl.*, Vol. 13, p. 591, 1912.

⁸ Lillie, Ralph S., *Am. Jour. Physiol.*, Vol. 24, p. 14, 1909.

⁹ Mast, Samuel O., "Light and the Behavior of Organisms, xi + 410 p., 1911.

¹⁰ Kanda, Sakyô, *Am. Jour. Physiol.*, Vol. 35, p. 162, 1914.

the same as described in the writer's previous paper.¹ The free-swimming larvæ, just after they leave the egg-strings at the swarming stage, are strikingly positive to light. They are, therefore, very favorable for work of this kind.

The methods of experimentation were simple. At each trial, four 100 c.c. beakers, one for control with normal sea-water at room temperature, a second with a treated sea-water at room temperature, a third with the same (treated) sea-water at higher temperature, and a fourth with the same (treated) sea-water at lower temperature, were used. Fifty c.c. of liquid were used in each beaker. Each beaker was set in a larger vessel (finger bowl), containing sea-water of the same temperature as the beaker. When all the beakers were ready and fairly constant at the desired temperatures, the larvæ taken with a small pipette from a very dense aggregation were distributed as uniformly as possible in each beaker.

Diffuse light came horizontally from one south window only. Care was taken against reflected light by using folded black cloth under the beakers and on a long board that was placed at the north side of the dishes.

III. EXPERIMENTAL.

Recent discoveries in the reversing effect of temperature of normal sea-water on the normal heliotropism of various animals had made it desirable to examine this phenomenon in *Arenicola* larvæ and to see whether there is any temperature coefficient when chemicals were added to it. In some experiments this was fairly illustrated, as far as time-relation was concerned, but others were not so successful as expected, possibly on account of the entrance of other factors.

I. *Effects of Temperature.*

Mast states that he did not succeed in producing a reversal of the positive heliotropism of *Arenicola* larvæ by changing the temperature of sea-water.² This was not the case in the writer's experiments. Mast perhaps used old larvæ instead of very

¹ Kanda, *loc. cit.*

² Mast, *loc. cit.*

young ones. The sensitivity of the larvæ to changes of medium varied greatly according to their age. It was impossible to get larvæ uniformly young. This was the reason why one could not get a reversal of all larvæ treated even with the best agents. There was always one or two per cent. of exceptions. Table I. gives the summary of several series of the writer's experiments.

TABLE I.
EFFECTS OF TEMPERATURE.

50 C.c. Sea-water.	Immed. After the Treatment.	1 Min. After the Treatment.	5 Min. After the Treatment.	10 Min. After the Treatment.	25 Min. After the Treatment.
31° C.	Practically all positive	Some became negative.	About 50 per cent. became negative.	Same as before.	Same as before.
11° C.	Almost motionless.	Many became positive.	Very few became negative.	About 15 per cent. became negative.	About 20 per cent. became negative.
21° C. (Control)	Practically all positive.	Practically all on the window side	Same as before.	Same as before.	A few became negative.

It was peculiar that high and low temperatures had the same reversing effect on the larvæ, although cold was slower and less effective. Loeb¹ showed in *Polygordius* larvæ a phenomenon similar to this.

2. Effects of Hypertonic and Hypotonic Sea-water.

(a) *Effect of Hypertonic Sea-water.*—R. S. Lillie² and Mast³ observed that hypertonic and hypotonic sea-water produced the reversal of the normal heliotropism in *Arenicola* larvæ. The writer tested the effect of hypertonic sea-water by mixing 15 c.c. of one mol NaCl solution and 35 c.c. of natural sea-water at room temperature, 22° C. He found that about 20 per cent. of larvæ became negative. He did not, however, succeed in producing a reversal in this medium by raising or lowering temperature, *i. e.*, 32° C. or 12° C. The larvæ in this mixture were very sluggish at these temperatures.

In the mixture of 0.85 c.c. of m-KCl solution and 50 c.c. of

¹ Loeb, J., *loc. cit.*

² Lillie, R. S., *loc. cit.*

³ Mast, S. O., *loc. cit.*

natural sea-water at room temperature, 22° C., it was found that 30 minutes after the treatment about 35 per cent. of the larvæ became negative to light. Besides sodium and potassium chlorides, m-CaCl₂, m-MgCl₂, and 2m-MgSO₄ were tested without success. Temperature experiments also failed in all these mixtures of hypertonic sea-water.

(b) *Effect of Hypotonic Sea-water.*—The mixture of 30 c.c. of natural sea-water and 20 c.c. of distilled water (which was the optimum dilution) was very remarkable in producing a reversal of heliotropism. The effect was temporary only. The results of several series are summarized in Table II. It is also interesting to note the difference in the time required to produce the maximum results at the three temperatures 12°, 22°, 32°. A Q₁₀ value of about 2 is indicated for the reversive process.

TABLE II.
EFFECT OF HYPOTONIC SEA-WATER.

Solution.	Temperature.	Best Result Obtained.	Result.
30 c.c. sea-water + 20 c.c. dist. water.	32° C.	10 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	22° C. (room temp.)	20 min. after the treatment.	About 90 per cent. became negative.
30 c.c. sea-water + 20 c.c. dist. water.	12° C.	40 min. after the treatment.	About 90 per cent. became negative.
50 c.c. nat. sea-water (control).	22° C. (room temp.)	1/2 min. after the treatment.	Practically all positive.

The larvæ lived over 15 days and grew well in this hypotonic sea-water.

3. *Effects of Isotonic Salt Solutions.*

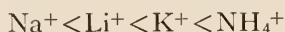
A question arose whether these reversing effects of hypertonic and hypotonic sea-water were simply osmotic or not. This idea was tested by using isotonic salt solutions. In all of these series the problem of temperature was omitted from consideration. The results are given in Table III.

Since the sodium, lithium, potassium or ammonium chloride and sulphate solutions that were used were all isotonic with sea-water, it is evident that the reversing effect of these salts is not simply an osmotic one. And since the action of these eight salts was different from that of calcium or magnesium chloride

TABLE III.
EFFECT OF ISOTONIC SALT SOLUTIONS.

Temp. of Mixt.	Mixture of Isot. Sol. and Sea-water.	Time After the Treatment for Maximum Effect.	Per Cent. of Negative Heliotropism Produced.
20° C.	1.5 c.c. 0.52 m. NH ₄ Cl + 50 c.c. sea-water.	About 1 hr.	About 90 per cent.
20° C.	2 c.c. 0.52 m. KCl + 50 c.c. sea-water.	About 20 min.	About 70 per cent.
21° C.	3 c.c. 0.54 m. LiCl + 50 c.c. sea-water.	About 25 min.	About 50 per cent.
21° C.	30 c.c. 0.52 m. NaCl + 20 c.c. sea-water.	About 1 hr.	About 30 per cent.
21° C.	20 c.c. 0.35 m. MgCl ₂ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	20 c.c. 0.35 m. CaCl ₂ + 30 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
20° C.	1 c.c. 0.46 m. (NH ₄) ₂ SO ₄ + 50 c.c. sea-water.	About 2 hrs.	About 90 per cent.
20° C.	1 c.c. 0.46 m. K ₂ SO ₄ + 50 c.c. sea-water.	About 40 min.	About 75 per cent.
21° C.	1.5 c.c. 0.47 m. Li ₂ SO ₄ + 50 c.c. sea-water.	About 1 hr.	About 70 per cent.
21° C.	20 c.c. 0.46 m. Na ₂ SO ₄ + 50 c.c. sea-water.	About 2 hrs.	About 60 per cent.
21° C.	12 c.c. 0.9 m. MgSO ₄ + 50 c.c. sea-water.	About 5 hrs.	Slight or indifferent.
21° C.	50 c.c. nat. sea-water.	About 1/2 m.-5 hrs.	Pract. all positive.

and sulphate solutions, the reversing effect of the former eight salts would seem to be specific. The chlorine and sulphate ions in these twelve salts being the same, the specific action of isotonic sodium, lithium, potassium or ammonium chloride and sulphate solutions seems to be due essentially to cations instead of anions. The effective order of these cations may be expressed as follows:



That a yellow pigment contained in the cells of the larvæ diffused out into the medium, when treated with the mixture of isotonic Na₂SO₄ or NH₄Cl solution and sea-water, was striking. This took place between eight and ten minutes after the treatment. A similar exit of pigment was first observed by Lillie in isotonic NaCl, KCl and NH₄Cl solutions, and has been generally regarded as indicating an increased permeability of the cell membrane.

A question arises whether the reversal of positive heliotropism of the larvæ was due directly to the diffusion of cations into the cells as indicated above or not. In the cases of Na₂SO₄ and

NH_4Cl solutions, the most notable exit of pigment from the larvæ—the sign of increased permeability according to Lillie—took place at about ten or twenty minutes after the treatment. In the case of Na_2SO_4 solution about 5 per cent. of the larvæ became negative at about ten minutes, while in NH_4Cl solution the larvæ were evenly distributed at about 20 minutes after the treatment. The maximum reversal in the former was produced at about two hours and in the latter at about one hour after the treatment. The reversing effect of these two salts, therefore, seems to be too slow to be regarded as the direct action of ions which have diffused into the larvæ. At the same time, the effect is not on the boundary membrane alone, because these salts must have modified and penetrated the membrane long before the change of reaction in the larvæ occurred. The larvæ treated with Na_2SO_4 and NH_4Cl stick tightly to the window side of a beaker 7 or 8 minutes after the treatment. This fact may explain the difficulty. That is to say, if they had been free in swimming, they might have reversed their heliotropic reaction more promptly than they actually did.

As to the way in which the behavior of organisms is modified by electrolytes such as salts, we are as yet in the dark. At any rate, the reversal of positive heliotropism of the larvæ by these electrolytes is an insufficient index by which to gain an understanding of the mechanism of their physical or chemical influence on the behavior of organisms.

4. *Effects of Artificial Sea-water.*

The artificial sea-water that the writer used was a van't Hoff mixture of the following composition:

0.52 m. NaCl	100	c.c.
0.52 m. KCl	2.2	c.c.
0.35 m. CaCl_2	1.5	c.c.
0.35 m. MgCl_2	7.8	c.c.
0.90 m. MgSO_4	3.8	c.c.

To this a trace of NaHCO_3 was added as Loeb advised. This artificial sea-water with or without the alkali affected little the positive heliotropism of the larvæ. About five per cent. of them showed negative heliotropism. Besides these, a few larvæ in

this solution swam about at the bottom of the beaker. About 90 per cent. or more of the larvæ were always positive to light.

5. *Effects of Hypertonic Sodium and Potassium Chloride Solutions Added to the Artificial Sea-water.*

These effects were remarkable. In the mixture of 15 c.c. m-NaCl and 35 c.c. of the artificial sea-water, about 90 per cent. of the larvæ became negative to light at about 20 minutes after the treatment. Most of the negative animals stayed at the bottom of the negative side of the beaker. In the mixture of 0.8 c.c. of m-KCl and 50 c.c. of the artificial sea-water, which was the optimum proportion, about 75 per cent. of the larvæ became negative to light at about 30 minutes after the treatment.

The mixture of 15 c.c. of isotonic NaCl and 35 c.c. of the artificial sea-water was also tested. It changed the reaction of about 85 per cent. of the larvæ. In comparing these results with those shown in Table III., it may be concluded that the effective order of ions is variable as other environmental conditions are varied. It is little wonder therefore that the order of ionic actions obtained by different investigators is not always uniform.

6. *Effects of Elimination of Certain Salts from the Artificial Sea-water.*

By eliminating each separate component from the artificial sea-water made up of six components, the specific rôle which such component played on either positive or negative heliotropism of the larvæ might be, the writer thought, in part understood.

First of all, NaCl solution was left out of the artificial sea-water. Larvæ transferred into the mixture without NaCl at 22° C., were practically all motionless immediately after the treatment. Sodium chloride being the essential component of the artificial sea-water as well as of the natural one, such result should be expected, since the osmotic as well as the chemical conditions are profoundly changed.

According to Ewald, however, "leaving NaCl out of artificial sea-water" "increases the negative and diminishes the positive" heliotropism of the nauplii of *Balanus perforatus* which are

naturally positive to light. Such was not the case in *Arenicola* larvæ, as just stated.

Elimination of KCl had a different effect. After transferring the larvæ into the artificial KCl-free sea-water at 23° C., practically all showed positive reaction immediately after the treatment, though a few soon became negative. About 20 minutes after the treatment, about 20 per cent. of all larvæ were at the top of the positive side of the beaker, about 30 per cent. at the bottom of the same side, and about 50 per cent. at the bottom of the negative side. If the larvæ collected at the bottom of the beaker farthest from the source of light could be called negative, one sees a puzzle hardly capable of solution. For, as already seen, addition of 2 c.c. of isotonic KCl solution to 50 c.c. of the of the natural sea-water also reversed about 70 per cent. of the larvæ. In other words, either addition or elimination of potassium salt produced a negativating effect on the larvæ. Besides potassium, however, there was another negativating component in the artificial sea-water, namely sodium. In the unbalanced artificial sea-water, sodium ions might exert a negativating effect on the larvæ.

In the calcium-free artificial sea-water at 23° C. all larvæ swam about at the bottom of the beaker. Their activity was much diminished; and about 15 minutes after the treatment a majority of them became almost motionless. CaCl_2 in the artificial sea-water as well as in the natural one is therefore essential to the life and activity of the larvæ, although of the five salts it is present in the smallest proportion. It is well known that calcium and magnesium antagonize the toxic action of sodium and potassium. Magnesium alone seemed, however, not to be sufficient in this case.

In the magnesium chloride-free or sulphate-free artificial sea-water at 23° C., immediately after the treatment practically all the larvæ were positive. About 10 minutes after the treatment about 60 per cent. or more of them were positive, about 20 per cent. negative, mostly at the bottom of the negative side of the beaker, and 20 per cent. or less were scattered at the bottom. During 1 to 5 hours after the treatment about 70 per cent. or less were positive, and the rest were at the bottom of the negative

side. When both $MgCl_2$ and $MgSO_4$ were left out at $23^\circ C.$, those larvæ which were at the bottom of the negative side constituted about 40 per cent. or more, whereas they were about 30 per cent. or more with elimination of either $MgCl_2$ or $MgSO_4$ alone. As has already been seen, the addition of magnesium chloride or sulphate to the natural sea-water had no negativating effect on the heliotropism of the larvæ. On the other hand, the addition of sodium or potassium chloride had such effect. The fact that magnesium and calcium chlorides seem to act as a positivating component in the artificial sea-water as well as in the natural sea-water is therefore clear, whereas potassium and sodium chlorides act as negativating ones. In other words, magnesium and calcium chlorides act in antagonizing the negativating effect of sodium or potassium chloride, or of both, although magnesium cannot antagonize the toxic action of the latter salts as calcium chloride can do, since the larvæ could not remain active and live long in calcium-free artificial sea-water.

7. *Effects of Alcohols.*

In narcotising *Arenicola* larvæ, Lillie used many narcotics. Of alcohols, he found that ethyl alcohol had a negativating effect on the heliotropism of the larvæ. It will be seen in Table IV. that all of the alcohols tested by the writer had more or less effect in making the larvæ negative to light. Difficulty was experienced, however, in getting uniform results in different series of experiments when different stocks of alcohols were used; probably this variability was largely due to impurity of reagents.

Just as Loeb has shown with copepods and *Daphnia*, it is obvious from Table IV. that the higher the alcohol in the series the greater negativating effect it had. This point will be considered a little later. With the exception of two cases, the higher the temperature the quicker and larger was the negativating effect produced. That the larvæ in the mixture of methyl or ethyl alcohol and natural sea-water at room temperature (22°) were less negative than at a temperature five degrees lower (17°) is hard to understand.

Acetone and two esters were also tested. The general features of the results obtained with these narcotics were quite similar to

TABLE IV.

EFFECTS OF ALCOHOLS.

Concentration of Alcohol Solution (made with Sea-water).	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		10-15 Min.	20-25 Min.	30 Min.
0.03 m. methyl alcohol.	32° C. 22° C. (R.T.) 17° C.	65 per cent.	15 per cent.	30 per cent.
0.016 m. ethyl alcohol.	32° C. 22° C. (R.T.) 17° C.	65 per cent.	25 per cent.	30 per cent.
0.002 m. n-propyl alcohol.	32° C. 22° C. (R.T.) 17° C.	70 per cent.	45 per cent.	35 per cent.
0.0006 m. n-butyl alcohol.	31° C. 21° C. (R.T.) 16° C.	70 per cent.	45 per cent.	35 per cent.

those obtained with alcohols. In no case, however, were the larvæ normal in behavior when the temperature was lowered either 5° C. or 10° C. below room temperature. On the other hand, production of negative heliotropism at the higher temperature (32°) was much more striking with the esters than with any of the alcohols. Table V. gives a summary of these series of experiments.

TABLE V.

EFFECTS OF ACETONE AND ESTERS.

Concentration of Narcotic Made with Sea-water.	Temperature.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.	
		10 Min.	20 Min.
0.01 m. acetone.	32° C. 22° C. (R.T.)	40 per cent.	25 per cent.
0.0045 m. ethyl acetate.	32° C. 22° C. (R.T.)	90 per cent.	30 per cent.
0.0025 m. ethyl acetate.	32° C. 22° C. (R.T.)	100 per cent.	45 per cent.

From Tables IV. and V. nothing certain can be concluded as to the temperature coefficient in the production of negative heliotropism, although the time needed to reach the maximum point is about halved by a rise of 10° C.

8. *Effect of Saponin.*

A solution of saponin made by adding 0.8 c.c. of 0.1 per cent. saponin to 50 c.c. of natural sea-water was also tried. The influence of temperature was well marked. At room temperature about 10 per cent. of the larvæ became negative after 30-40 minutes in this solution: at 10° above room temperature about 90 per cent. became negative within 20 minutes.

9. *Effects of Fatty Acids.*

“Loeb hat . . . quantitative Versuche über die zur Erzeugung von positivem Heliotropismus bei Daphnien und Copepoden nötige Konzentration verschiedener Säuren und Alkohole gemacht. Die folgende Tabelle gibt die minimale Konzentration für die Erregung von positivem Heliotropismus bei Copepoden für verschiedene Säuren und Alkohole:

Ameisensäure.....	0.006	N.
Essigsäure.....	0.006	“
Propionsäure.....	0.005	“
Buttersäure.....	0.004	“
Valeriansäure.....	0.004	“
Capronsäure.....	0.0019	“
Aethylalkohol.....	0.19	“
Propylalkohol.....	0.054	“
Normaler Butylalkohol.....	0.019	“
Amylalkohol.....	0.011	“

Man sieht, dass die Wirksamkeit der Alkohole sehr rasch mit steigender Zahl der Kohlenstoffatome zunimmt, während bei den Säuren der Zuwachs nur eben merklich ist. Die Folge ist, dass, während die niedrigen Alkohole viel weniger wirksam sind als die korrespondierenden Fettsäuren, bei den höheren Gliedern dieser Unterschied verschwindet.”¹

In comparing the results obtained by Loeb with those which are given in Tables IV. and VI. a general agreement will be found. It is also worth mentioning that the reversal of the larvæ in acidified sea-water took place immediately after the treatment at a temperature 10° C. higher than room temperature, whereas it took place about two minutes after the treatment at room temperature. In this experiment and others shown in

¹ Loeb, Jacques, “Handbuch d. Vergl. Physiol.,” Bd. IV., Erste Hälfte, S. 470.

Table VI., the temperature coefficient (Q_{10}) of the maximum reversal of the normal heliotropism appeared to be between 2 and 3. The reversal may, therefore, be interpreted as the result of a chemical reaction. The marked physiological activity of the fatty acids seems to depend largely on their lipid-solubility. At lowered temperatures their effectiveness in producing negative heliotropism is somewhat lessened, as Table VI. shows.

TABLE VI.
EFFECTS OF FATTY ACIDS.

Concentration of Fatty Acid Solution (Made with Sea-water).	Temperature, Degrees C.	Maximum Per Cent. of Negative Heliotropism Produced After the Treatment.		
		5 Min.	10-15 Min.	20-30 Min.
0.0025 m. formic acid.	32	90 per cent.		
	22 (R. T.)		85 per cent.	
	12			20 per cent.
0.0010 m. acetic acid.	32	95 per cent.		
	22 (R. T.)		90 per cent.	
	12			30 per cent.
0.0015 m. propionic acid.	32	95 per cent.		
	22 (R. T.)		90 per cent.	
	12			60 per cent.
0.0011 m. butyric acid.	32	95 per cent.		
	22 (R. T.)		95 per cent.	
	12			90 per cent.
0.00075 m. valeric acid.	31 $\frac{1}{2}$	95 per cent.		
	21 $\frac{1}{2}$ (R. T.)		95 per cent.	
	11 $\frac{1}{2}$			20 per cent.
0.00006 m. caproic acid.	31 $\frac{1}{2}$	95 per cent.		
	21 $\frac{1}{2}$ (R. T.)		95 per cent.	
	11 $\frac{1}{2}$			

Of all chemicals tested by the writer, fatty acids were the best for reversing normal heliotropism. If one reversed the heliotropism of a mass of larvæ by fatty acid, a large percentage gradually accumulated at the side of the dish away from the light. If now one turned the dish through 180°, it was most striking to see the hosts of larvæ quickly assume the new orientation and swim to the negative side. Their general behavior and activity appeared normal. Many, however, swam at the bottom of the negative side of the dish, especially in solutions of the higher fatty acids. Curiously enough, this efficiency of fatty acids as a reverser of heliotropism runs closely parallel to their physiological efficiency in producing artificial parthenogenesis, as discovered by Loeb.

10. "*Antagonism*" *Between Acid and Narcotics.*

It is known through the work of R. Lillie that an antagonism between salts and narcotics is readily demonstrated in *Arenicola* larvæ. In the present investigation it was found that chloroform, ethyl alcohol and ether had little or no negativating effect on the larvæ at room temperature; on the other hand, butyric acid proved to be the most effective of all the negativating agents employed. The writer thought therefore that antagonism might be demonstrated in the mixture of any of those narcotics and butyric acid. It was found that the negativating effect of butyric acid was much retarded in the presence of a narcotic. In sea-water containing .0022 m. butyric acid 95 per cent. of the larvæ became negative after 15 minutes exposure. In the same solution to which chloroform, ether, or formaldehyde were added in appropriate concentrations no reversal was evident after 15 minutes and only 50 per cent. were negative after one hour's exposure, as compared with 90-100 per cent. in the control. Apparently the process of reversal is retarded in the presence of the anæsthetic.

11. *Effect of Solutions of Butyric Acid made with Artificial Sea-water.*

The writer tested the effect of butyric acid in artificial sea-water. It was found that a very weak concentration of butyric acid, *i. e.*, 0.00006 mol., was efficient in negativating about 80 per cent. of the larvæ in about 10 minutes after the treatment.

12. *Effects of Inorganic Acids.*

Loeb¹ found that the monobasic fatty acids were very effective, whereas strong acids, such as HCl, HNO₃ and H₂SO₄ "had so little effect as to be practically useless" for experiments on the membrane formation of sea-urchin eggs in artificial parthenogenesis. He concludes that this proves a diffusion of undissociated molecules into cells and denies an increased permeability of the cells for hydrogen ions.² The writer tested this idea in producing the negative heliotropism of larvæ, but the results

¹ Loeb, Jacques, *Biochem. Zeits.*, Bd. 15, S. 254, 1909; "Artificial Parthenogenesis and Fertilization," p. 133, 1913.

² Loeb, J., *loc. cit.*, pp. 142-143.

obtained by the writer did not agree well with those of Loeb, as in the case of fatty acids. For instance, in 0.0036 m. HCl solution made with sea-water, about 80 per cent. of the larvæ became negative and in similar solutions of H_2SO_4 about 95 per cent. became negative 15 minutes after the treatment at $21^\circ C$. The negative larvæ, however, showed marked abnormality. This was the only case of failure, so far as the writer found, in the parallelism between the effects of chemical substances in producing artificial parthenogenesis in sea-urchin eggs and in reversing the positive heliotropism of *Arenicola* larvæ.

The writer also found at the Marine Biological Laboratory of the Kyushu Imperial University, Fukuoka, Japan, in the summer of 1916, that a marine crustacean, *Astracoda Cypridina* (*hilgendorfi*) which was strongly negative to light, could be made positive by HCl or H_2SO_4 solution. Quantitative data were not obtained owing to illness.

That acids, organic and inorganic, are able to penetrate the living cell, is proved by Harvey.³ It seems possible therefore that both HCl and H_2SO_4 penetrated into *Arenicola* larvæ.

13. *Effects of Strong and Weak Bases.*

Loeb discovered that "the weak base NH_4OH is much more efficient for the causation of artificial parthenogenesis in *Arbacia* eggs than the strong bases, NaOH, KOH and tetraethylammonium hydroxide,"¹ $N(C_2H_5)_4OH$. The writer found that this was also the case in the production of negative heliotropism in *Arenicola* larvæ. The essential results of his experiments may be summarized thus: in the appropriate concentration of NH_4OH , about 70 per cent. of the larvæ became negative after about 20 minutes exposure to the solution at $21^\circ C$., whereas in equivalent solutions of NaOH, KOH, or $N(C_2H_5)_4OH$, only about 10 or 15 per cent. of the larvæ became negative after an exposure of about 60 minutes at this temperature. This difference may be explained by the fact that the living cell is permeable to ammonium hydroxide but impermeable to sodium hydroxide and other strong bases, as experiments of Bethe² and Warburg³ have

¹ Harvey, E. Newton, *Science*, N. S., Vol. 39, p. 947, 1914.

² Loeb, Jacques, *Jour. Ex. Zoöl.*, Vol. 13, p. 577, 1912.

³ Bethe, Albrecht, *Pflüger's Arch.*, Bd. 127, S. 219, 1909.

⁴ Warburg, Otto, *Zeits. physiol. Chem.*, Bd. 66, S. 305, 1910.

clearly shown. Possibly the weak base NH_4OH is more effective than the stronger bases because of greater solubility in the lipoids of the plasma membranes.

14. *Effects of Chemicals after Returning into Normal Sea-water.*

Lillie observed one curious effect in larvæ treated with one fourth and one fifth saturated solutions of chloroform in sea water; when the larvæ, after about two hours in corked flasks containing these solutions, were poured out into watch glasses the great majority were found, after three or four hours (when the chloroform had evaporated), to exhibit a pronounced negative phototaxis in place of the usual positive. . . .¹ As was pointed out in his previous paper, the writer found a similar phenomenon in the larvæ treated with sodium, potassium, calcium and magnesium chloride solutions when they were returned into normal sea-water.² This was particularly remarkable in the larvæ treated with the last two solutions during the actual immersion in which there was never any reversal of positive heliotropism.

In the present experiments, the same phenomenon was also found in larvæ treated with salts, narcotics—chloroform, chloral and formalin in particular—strong alkalis, potassium cyanide, etc. As already shown, most of these chemicals were not favorable agents for the reversal of the positive heliotropism of the larvæ. Moreover, in some cases, KCN, for instance, the larvæ remained positive or indifferent to light while in presence of the chemical substance (dissolved in normal sea-water). Nevertheless the "after-effect" was pronounced. That is to say, almost all larvæ became negative when returned to normal sea-water.

Such facts are consistent with the hypothesis that these chemicals act by changing in some way the normal properties of the plasma membranes of the irritable elements concerned, so that a certain time is required to recover normal conditions after return to normal sea-water.

¹ Lillie, R. S., *loc. cit.*, p. 34.

² Kanda, S., *loc. cit.*

³ Lillie, R. S., *loc. cit.*, p. 34.

IV. SUMMARY.

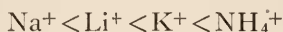
1. At room temperature nearly all *Arenicola* larvæ in the early swarming stage in normal sea-water are positively heliotropic. At a temperature 10° C. higher (30°–34°) about half of the larvæ become negative to light after about 5 minutes' exposure. At 10° C. lower than room temperature (ca. 12°), about 20 per cent. of the larvæ become negative after about 25 minutes exposure.

2. In sea-water made hypertonic by addition of NaCl or KCl some larvæ (20–35 per cent.) become negative.

3. In sea-water made hypertonic with CaCl₂, MgCl₂ or MgSO₄, no reversal was observed.

4. In hypotonic sea-water, about 90 per cent. of the larvæ reversed their heliotropism; reversal takes place more rapidly the higher the temperature (between 12° and 32°).

5. Isotonic solutions of ammonium, potassium, lithium, sodium chlorides and sulphates made in sea-water reverse the positive heliotropism of the larvæ. But isotonic solutions of magnesium and calcium chlorides and sulphates in sea-water do not. The reversing effect of these salts seems, therefore, due to the cations. This suggests an action on the plasma membranes (*cf.* the membrane theory of Ostwald-Bernstein).¹ The effective order of these cations is



6. In artificial sea-water, the positive heliotropism of the larvæ is not noticeably different from that in natural sea-water. A trace of alkali makes apparently no difference.

7. Hypertonic sodium and potassium chloride solutions added to the artificial sea-water reversed the positive heliotropism of the majority of the larvæ.

8. In sodium-free artificial sea-water, the larvæ became motionless immediately after the treatment (effect of hypotony).

9. In potassium-free artificial sea-water, many became negative to light, and remained at the bottom of the negative side of the beaker.

10. In calcium-free artificial sea-water, the larvæ became motionless about 15 minutes after the exposure. Magnesium

¹ Bernstein, J., *Pflüger's Arch.*, Bd. 122, S. 129.

alone, therefore, cannot antagonize the toxic action of sodium and potassium salts.

11. In the magnesium-free artificial sea-water, many remain positive to light. This fact may be explained as an antagonistic action of calcium toward sodium and potassium salts which acting by themselves are negativating agents.

12. Alcohols (methyl, ethyl, n-propyl, n-butyl) as used by the writer are not favorable as negativating agents at room temperature, though they are good at higher temperature. The higher the alcohol in the series the greater negativating effect it has.

13. Most of the larvæ treated with esters, methyl and ethyl acetate, became negative at higher than room temperature.

14. Other compounds, chloroform, formalin, ether and saponin, produced little or no reversal while the larvæ were in the solutions, except saponin at higher temperatures.

15. Monobasic fatty acids are the best of all chemicals used for this work. The higher the acid in the series the greater negativating effect it has.

16. Some narcotics "antagonize" or retard the reversing action of butyric acid.

17. The reversing effect of a very weak concentration of butyric acid (.00006 m.) in artificial sea-water is great.

18. Hydrochloric and sulphuric acids produce negative heliotropism, almost as well as fatty acids do.

19. In producing negative heliotropism, NH_4OH is much more efficient than strong bases.

20. *Arenicola* larvæ treated with narcotics become negative after returning into normal sea-water.